UNIVERSIDADE FEDERAL DE PERNAMBUCO

CENTRO ACADÊMICO DA VITÓRIA

BEATRIZ MACHADO SILVA

AVALIAÇÃO DOS EFEITOS IN VIVO DO P-MAPA, SILIMARINA E PRAZIQUANTEL EM OVOS E VERMES PRESENTES NOS TECIDOS HEPÁTICO E INTESTINAL DE CAMUNDONGOS INFECTADOS EXPERIMENTALMENTE POR *Schistosoma mansoni*

VITÓRIA DE SANTO ANTÃO

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NÚCLEO DE ENFERMAGEM

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Orientador: Dr. José Candido de Souza Ferraz Junior
Coorientador: Dr. Fábio Lopes de Melo e MsC. Rhaíssa Evelyn Moraes Ramos

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Aprovado em: 15/12/2021.

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RESUMO

A esquistossomose é uma parasitose causada por trematódeos do gênero Schistosoma. No Brasil, cerca de 1,5 milhões de pessoas vivem em áreas vulneráveis à infecção pelo helminto. O fármaco de primeira escolha para o tratamento da esquistossomose é o praziquantel, que possui diversas limitações. O agregado polimérico de fosfolinoleato-palmitoleato de magnésio e amônio proteico (P-MAPA) e a silimarina são potenciais adjuvantes no tratamento desta parasitose devido às suas propriedades imunomoduladoras e regeneradoras. Desta forma, o objetivo do presente estudo foi avaliar os efeitos in vivo do P-MAPA, silimarina e praziquantel, isolados ou combinados, em ovos presentes nos tecidos hepático e intestinal de camundongos infectados experimentalmente por Schistosoma mansoni durante a fase crônica. O estudo foi desenvolvido com camundongos Swiss Webster, que foram infectados com aproximadamente 80 cercárias de Schistosoma mansoni por animal, através da imersão caudal, sob luz incandescente por uma hora. Os protocolos de tratamentos adotados foram: a) Praziquantel a 50 mg/kg/5 dias consecutivos por gavagem; b) P-MAPA 100mg/kg/dose única via intraperitoneal; c) e Silimarina a 10 mg/kg a cada 48h por 10 doses, via gavagem. Todos os grupos tratados com praziquantel reduziram o quantitativo de ovos nos tecidos. O grupo tratado com praziquantel e silimarina apresentou redução acima de 83% dos ovos no tecido hepático. O grupo tratado com praziquantel e P-MAPA apresentou 83,91% dos ovos mortos. A combinação das três drogas demonstrou resultados satisfatórios (71,5%), o que pode indicar uma promissora ferramenta terapêutica contra a esquistossomose e os danos causados pela doença.

Palavras-chave: esquistossomose; fitoterapia; doenças negligenciadas.

ABSTRACT

Schistosomiasis is a parasitosis caused by trematodes of the genus Schistosoma. In Brazil, around 1.5 million people live in areas vulnerable to the parasite infection. The drug of first choice for the treatment of schistosomiasis is praziquantel, which has several limitations. The polymeric aggregate of phospholinoleate-palmitoleate of magnesium and ammonium protein (P-MAPA) and silymarin are potential adjuvants in the treatment of this parasitosis due to their immunomodulating and regenerating properties. Thus, the aim of the present study was to evaluate the in vivo effects of P-MAPA, silymarin and praziquantel, isolated or combined, on the oviposition of Schistosoma mansoni eggs in the liver and intestine of mice experimentally infected in the chronic phase. The study was carried out with Swiss Webster mice, which were infected with approximately 80 cercariae of Schistosoma mansoni per animal, through caudal immersion under incandescent light for one hour. The treatment protocols adopted were: a) Praziquantel at 50 mg/kg/5 consecutive days by gavage; b) P-MAPA 100mg/kg/single dose intraperitoneally; c) and silymarin at 10 mg/kg every 48 hours for 10 doses, via gavage. All groups treated with praziquantel reduced the amount of eggs in tissues. The group treated with praziquantel and silymarin showed a reduction above 83% of eggs in liver tissue. The group treated with praziquantel and P-MAPA had 83.91% of dead eggs. The combination of the three drugs showed satisfying results (71,5%), which may indicate a promising therapeutic tool against schistosomiasis and the damage caused by the disease.

Keywords: Schistosomiasis; Phytotherapy; Neglected Diseases.

SUMÁRIO

1 INTRODUÇÃO

A esquistossomose é uma doença negligenciada causada por trematódeos do gênero Schistosoma, de ocorrência endêmica em regiões tropicais e subtropicais do planeta, como Egito, Venezuela e Brasil (WHO, 2021). No Brasil, cerca de 1,5 milhões de pessoas vivem em áreas vulneráveis à infecção pelo parasita, nas quais o Nordeste e o Sudeste se destacam como as regiões mais afetadas (DA SILVA et al., 2019; BRASIL, 2019). Esta parasitose é transmitida através do contato do ser humano com cercárias em coleções hídricas de poucas correntezas como córregos e lagoas (BRASIL, 2018). A esquistossomose pode provocar complicações como hemorragia digestiva, hipertensão portal e até mesmo a morte (ZAGHLOUL, M. et al., 2020; MAWA et al., 2021).

O tratamento é feito através da quimioterapia em massa com o praziquantel, que possui limitações como baixa eficácia contra o verme jovem, efeitos adversos graves dependendo da intensidade da infecção e da quantidade de doses administradas devido a baixa solubilidade (SILVA et al., 2017) e relatos de resistência do verme à droga (STELMA et al., 1995). Além disso, a alta prevalência de reinfecção, repetição do tratamento devido à falta de opções na indústria farmacêutica (ADEKIYA et al., 2020) e o sabor amargo do medicamento (MEYER et al., 2009) dificultam ainda mais a adesão ao tratamento por parte dos usuários.

Portanto, devido às restrições na eficácia do praziquantel é necessário o desenvolvimento de novas drogas capazes de assumir um papel adjuvante no tratamento da esquistossomose (TA et al., 2020). O agregado polimérico de fosfolinoleato-palmitoleato de magnésio e amônio proteico (P-MAPA) é um imunomodulador desenvolvido pela Farmabrasilis através da fermentação do fungo Aspergillus oryzae. O P-MAPA apresentou eficácia significativa quando utilizado em estudos no tratamento de doenças como tuberculose (FÁVARO et al., 2012) e leishmaniose visceral (SANTIAGO et al., 2013). A droga foi comprovadamente capaz de estimular a proliferação de linfócitos T e citocinas, entre elas a Interleucina-2 (IL-2). Devido a sua versatilidade e mínima toxicidade ((JCS et al., 2021), o imunomodulador P-MAPA se tornou promissor no combate às doenças infecciosas e imunossupressoras como cânceres de bexiga e de ovário (FÁVARO et al., 2012; (LUPI JÚNIOR et al., 2019).

A silimarina é o extrato da Silybum marianum (MARMOUZI et al., 2021), uma planta medicinal tradicionalmente utilizada há mais de 2.000 anos no tratamento de distúrbios hepáticos (WANG; ZHANG; WU, 2020). Este fitoterápico é composto por silibina, isosilibinina, silicristina, isossilicristina, silidianina e taxifolina. Dentre essas substâncias, a silibina é o componente que está presente em maior quantidade, correspondendo a cerca de 70% da composição total (FEDERICO et al., 2017). A silimarina possui atividade regeneradora, hepatoprotetoras, antioxidante, anti-inflamatória (HEIDARIAN, 2021), renoprotetora, neuroprotetora, hipoglicemiante, antitumoral e imunomodulatória (MARMOUZI et al., 2021), além de neutralizar a toxicidade de antibióticos, metais e pesticida (WANG; ZHANG; WU, 2020). A silibina foi comprovadamente capaz de modular a inflamação, desativando os sinais pró-inflamatórios que derivam do complexo proteico NF-κB, além de induzir a apoptose através modulação os níveis de proteína 2 do linfoma de células B (BCL-2) e proteína X associada a BCL-2 (BAX) em camundongos (FEDERICO et al., 2017).

Diante do exposto, a continuidade dos estudos envolvendo drogas já conhecidas, como a silimarina, e a aplicação de novas drogas, como o P-MAPA, são de extrema importância para que efeitos terapêuticos mais promissores no tratamento desta parasitose possam ser descobertos. Opções de tratamento que sejam capazes de melhorar a resposta imunológica do hospedeiro frente ao Schistosoma mansoni, além de tratar as consequências geradas pela esquistossomose se fazem necessárias. Nesse sentido, o P-MAPA e a silimarina se tornam potenciais adjuvantes no tratamento da esquistossomose devido às suas propriedades imunomoduladoras e regeneradoras. Dessa forma, o objetivo do estudo foi avaliar os efeitos in vivo do P-MAPA, silimarina e praziquantel, isolados ou combinados, na oviposição e nos vermes adultos de Schistosoma mansoni no fígado e intestino de camundongos na fase crônica da esquistossomose.

2 REVISÃO DE LITERATURA

2.1 O Schistosoma mansoni e a Esquistossomose

A esquistossomose é uma doença negligenciada causada por trematódeos do gênero Schistosoma (*S. mansoni, S. haematobium, S. japonicum, S. intercalatum, S. mekongi e S. guineenses*) e notificada em pelo menos 78 países (WHO, 2021), dentre eles Egito, Venezuela e Brasil (BRASIL, 2019). De acordo com a Organização Mundial da Saúde (2021), estima-se que cerca de 236,6 milhões necessitaram de tratamento preventivo no ano de 2019. No Brasil, cerca de 1,5 milhões de pessoas vivem em áreas vulneráveis à infecção do parasita, nas quais o Nordeste e o Sudeste se destacam como as regiões mais afetadas (BRASIL, 2019).

No Brasil, destacam-se os estados de Sergipe, Pernambuco, Alagoas, Minas Gerais e Bahia como os mais alarmantes, de acordo com o Inquérito Nacional de Prevalência da Esquistossomose e das Geo-helmintoses, realizado no período de 2010 a 2014 (KATZ, 2018). Em Pernambuco, há casos registrados em 102 municípios, a maior parte localizada nas regiões da Zona da Mata, Agreste e Região Metropolitana do Recife (RMR) (BRASIL, 2011; OLIVEIRA et al., 2018).

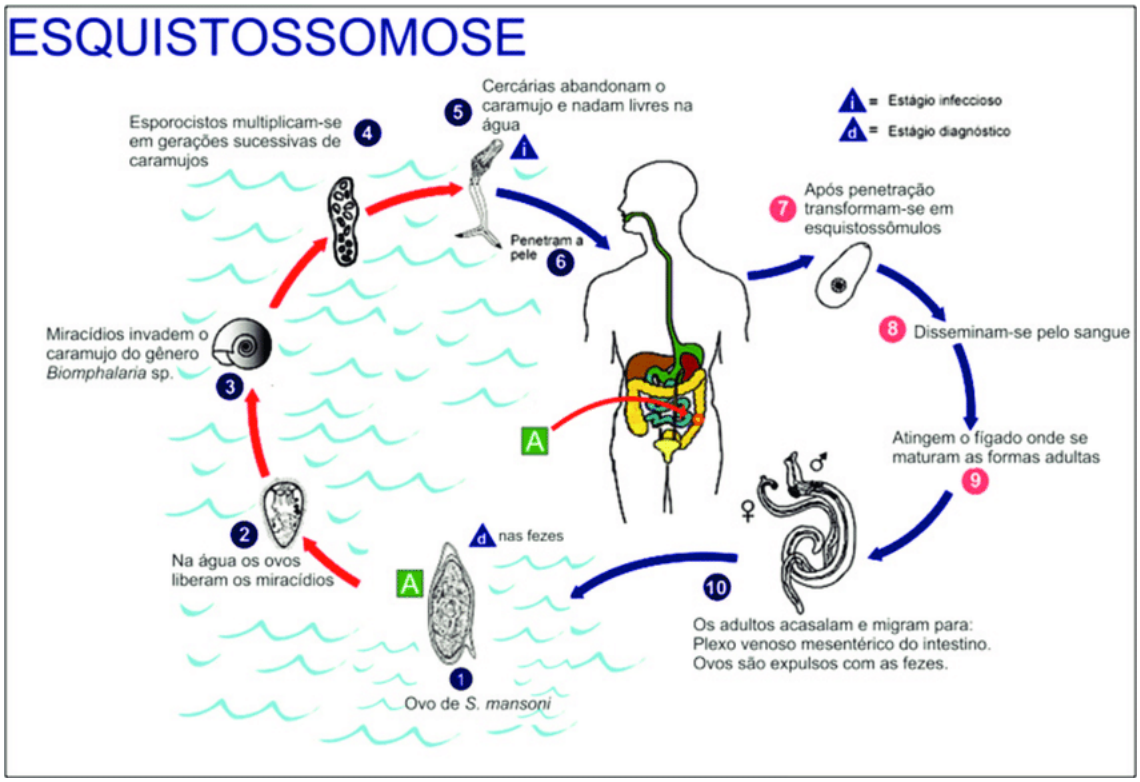
A doença possui estágio agudo e o crônico e as manifestações clínicas vão variar de acordo com a fase do desenvolvimento do verme e do local de alojamento no organismo do hospedeiro (BRASIL, 2019). A esquistossomose, quando não tratada precocemente, pode provocar complicações como hemorragia digestiva, hipertensão portal e até mesmo a morte (MAWA et al., 2021).

A prevenção contra a esquistossomose pode ser feita através algumas estratégias como a educação em saúde e a quimioterapia profilática, já que nenhuma vacina contra a doença foi aprovada até o momento (WILSON, 2020). Diversos fatores sanitários e socioeconômicos impedem a erradicação da parasitose como condições precárias de moradia, saneamento básico e abastecimento de água, falta de acesso à educação, crescimento urbano desordenado, remuneração inadequada e hábitos culturais que favorecem a propagação do verme (BRASIL, 2018).

2.2 Ciclo Biológico e Transmissão

O *Schistosoma Mansoni* possui um ciclo de vida heteroxênico. O hospedeiro intermediário desse parasito é o molusco do gênero *Biomphalaria (B. glabrata, B. straminea e B. tenagophila)*, que libera as cercárias em coleções hídricas que pouca correnteza, onde nadam em busca do hospedeiro definitivo para que o ciclo se complete (BRASIL, 2008).

Figura 1 – Ciclo biológico do S. mansoni (adaptado CDC)



Fonte: Center for Disease Control and Prevention (traduzido por GOMES; DOMINGUES; BARBOSA, 2017**.**

A transmissão do *S. mansoni* tem inicio quando o homem elimina fezes contaminadas na água. Os ovos medem medem cerca de 150µm de comprimento x 65 µm de largura e possuem um espículo lateral voltado para trás. Quando o ovo está maduro, o míracidio pode ser visualizado no seu interior graças à transparência da casca.

Figura 4 - Ovo de *S. mansoni* maduro com miracídio em seu interior

Fonte: GOMES; DOMINGUES; BARBOSA, 2017.

Ao entrarem em contato com a água, os ovos eclodem e liberam o miracídio. Esta fase evolutiva apresenta uma forma cilíndrica com superfície ciliada e possui o terebratorium na extremidade anterior, que auxiliam na fixação ao caramujo. Ao penetrar no caramujo, o miracídio se diferencia em esporocisto e sofre enúmeras transformações e multiplicações, até atingirem o estágio de cercária (REY, 2015). As cercárias (Figura 2) possuem uma cauda bifurcada muscular, um corpo ovoide e achatado, e uma cabeça com duas ventosas (COLLEY et al., 2014). Os caramujos liberam as cercarias no horário de maior incidência solar, entre 11h e 15h.

Figura 2 - Cercária de *S. mansoni*

Fonte: GOMES; DOMINGUES; BARBOSA, 2017.

Após penetrarem na pele do humano, as cercárias perdem a cauda e se tornam esquistossômulos. O esquistossômulo, então, migra da pele para a circulação até chegar ao sistema porta intra-hepático. Chegando lá, o verme jovem irão se alimentar e se tornar verme adulto, seja ele macho ou fêmea (MELO; COELHO, 2005).Ao atingirem a fase adulta, os vermes acasalam e migram, juntos, até as veias mesentéricas inferiores, onde liberam ovos imaturos nos capilares venosos que irrigam o intestino. Os ovos depositados nos tecidos se tornam maduros após uma semana (miracídio formado) e, em seguida, metade dos ovos vão para luz intestinal e são eliminados nas fezes do hospedeiro definitivo, dando início a um novo ciclo (MELO; COELHO, 2005). A outra metade permanece nos tecidos e desencadeia uma resposta imune mediada por células Th2 e a formação de granuloma ao redor dos ovos nos tecidos do hospedeiro (SCHWARTZ; FALLON, 2018).

3 ARTIGO

O presente trabalho está apresentado no formato de artigo requerido pela revista **Experimental Parasitology**, cujas normas para submissão de artigos se encontram em anexo.

AVALIAÇÃO DOS EFEITOS *IN VIVO* DO P-MAPA, SILIMARINA E PRAZIQUANTEL EM OVOS E VERMES PRESENTES NOS TECIDOS HEPÁTICO E INTESTINAL DE CAMUNDONGOS INFECTADOS EXPERIMENTALMENTE POR *Schistosoma mansoni*

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Conflito de interesse: Nenhum.

RESUMO

A esquistossomose é uma parasitose causada por trematódeos do gênero *Schistosoma*. No Brasil, cerca de 1,5 milhões de pessoas vivem em áreas vulneráveis à infecção pelo helminto. O fármaco de primeira escolha para o tratamento da esquistossomose é o praziquantel, que possui diversas limitações. O agregado polimérico de fosfolinoleato-palmitoleato de magnésio e amônio proteico (P-MAPA) e a silimarina são potenciais adjuvantes no tratamento desta parasitose devido às suas propriedades imunomoduladoras e regeneradoras. Desta forma, o objetivo do presente estudo foi avaliar os efeitos *in vivo* do P-MAPA, silimarina e praziquantel, isolados ou combinados, em ovos presentes nos tecidos hepático e intestinal de camundongos infectados experimentalmente por *Schistosoma mansoni* durante a fase crônica. O estudo foi desenvolvido com camundongos Swiss Webster, que foram infectados com aproximadamente 80 cercárias de *Schistosoma mansoni* por animal, através da imersão caudal, sob luz incandescente por uma hora. Os protocolos de tratamentos adotados foram: a) Praziquantel a 50 mg/kg/5 dias consecutivos por gavagem; b) P-MAPA 100mg/kg/dose única via intraperitoneal; c) e Silimarina a 10 mg/kg a cada 48h por 10 doses, via gavagem. Todos os grupos tratados com praziquantel reduziram o quantitativo de ovos nos tecidos. O grupo tratado com praziquantel e silimarina apresentou redução acima de 83% dos ovos no tecido hepático. O grupo tratado com praziquantel e P-MAPA apresentou 83,91% dos ovos mortos. A combinação das três drogas demonstrou resultados satisfatórios (71,5%), o que pode indicar uma promissora ferramenta terapêutica contra a esquistossomose e os danos causados pela doença.

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Keywords**:** Schistosomiasis. Phytotherapy. Neglected Diseases.

1 INTRODUÇÃO

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O tratamento é feito através da quimioterapia em massa com o praziquantel, que possui limitações como baixa eficácia contra o verme jovem, efeitos adversos graves dependendo da intensidade da infecção e da quantidade de doses administradas devido a baixa solubilidade (SILVA et al., 2017) e relatos de resistência do verme à droga (STELMA et al., 1995). Além disso, a alta prevalência de reinfecção, repetição do tratamento devido à falta de opções na indústria farmacêutica (ADEKIYA et al., 2020) e o sabor amargo do medicamento (MEYER et al., 2009) dificultam ainda mais a adesão ao tratamento por parte dos usuários.

Portanto, devido às restrições na eficácia do praziquantel é necessário o desenvolvimento de novas drogas capazes de assumir um papel adjuvante no tratamento da esquistossomose (TA et al., 2020). O agregado polimérico de fosfolinoleato-palmitoleato de magnésio e amônio proteico (P-MAPA) é um imunomodulador desenvolvido pela Farmabrasilis através da fermentação do fungo Aspergillus oryzae. O P-MAPA apresentou eficácia significativa quando utilizado em estudos no tratamento de doenças como tuberculose (FÁVARO et al., 2012) e leishmaniose visceral (SANTIAGO et al., 2013). A droga foi comprovadamente capaz de estimular a proliferação de linfócitos T e citocinas, entre elas a Interleucina-2 (IL-2). Devido a sua versatilidade e mínima toxicidade ((JCS et al., 2021), o imunomodulador P-MAPA se tornou promissor no combate às doenças infecciosas e imunossupressoras como cânceres de bexiga e de ovário (FÁVARO et al., 2012; (JÚNIOR et al., 2019).

A silimarina é o extrato da Silybum marianum (MARMOUZI et al., 2021), uma planta medicinal tradicionalmente utilizada há mais de 2.000 anos no tratamento de distúrbios hepáticos (WANG; ZHANG; WU, 2020). Este fitoterápico é composto por silibina, isosilibinina, silicristina, isossilicristina, silidianina e taxifolina. Dentre essas substâncias, a silibina é o componente que está presente em maior quantidade, correspondendo a cerca de 70% da composição total (FEDERICO et al., 2017). A silimarina possui atividade regeneradora, hepatoprotetoras, antioxidante, anti-inflamatória (HEIDARIAN, 2021), renoprotetora, neuroprotetora, hipoglicemiante, antitumoral e imunomodulatória (MARMOUZI et al., 2021), além de neutralizar a toxicidade de antibióticos, metais e pesticida (WANG; ZHANG; WU, 2020). A silibina foi comprovadamente capaz de modular a inflamação, desativando os sinais pró-inflamatórios que derivam do complexo proteico NF-κB, além de induzir a apoptose através modulação os níveis de proteína 2 do linfoma de células B (BCL-2) e proteína X associada a BCL-2 (BAX) em camundongos (FEDERICO et al., 2017).

Diante do exposto, a continuidade dos estudos envolvendo drogas já conhecidas, como a silimarina, e a aplicação de novas drogas, como o P-MAPA, são de extrema importância para que efeitos terapêuticos mais promissores no tratamento desta parasitose possam ser descobertos. Opções de tratamento que sejam capazes de melhorar a resposta imunológica do hospedeiro frente ao Schistosoma mansoni, além de tratar as consequências geradas pela esquistossomose se fazem necessárias. Nesse sentido, o P-MAPA e a silimarina se tornam potenciais adjuvantes no tratamento da esquistossomose devido às suas propriedades imunomoduladoras e regeneradoras. Dessa forma, o objetivo do estudo foi avaliar os efeitos in vivo do P-MAPA, silimarina e praziquantel, isolados ou combinados, na oviposição e nos vermes adultos de Schistosoma mansoni no fígado e intestino de camundongos na fase crônica da esquistossomose.

2 MATERIAIS E MÉTODOS

2.1 População do estudo

O estudo foi desenvolvido com camundongos albinos machos, Swiss Webster, pesando entre 28-30 gramas, com 28 dias de idade, provenientes do biotério do

Instituto Aggeu Magalhães (IAM) da Fundação Oswaldo Cruz (FIOCRUZ). Os animais foram alocados em micro-isoladores em estantes ventiladas e ambiente controlado com ciclo claro/escuro (12h/12h), temperatura controlada em cerca de 22 °C, ração específica para roedores e água ad libitum. O Estudo foi aprovado (197/2018) pelo Comitê de Ética no Uso de Animais do IAM.

2.2 Infecção dos animais

Os camundongos foram infectados com aproximadamente 80 cercárias de Schistosoma mansoni por animal, através da imersão caudal, sob luz incandescente por uma hora. As cercárias foram obtidas de diferentes caramujos previamente infectados e cedidos pelo Laboratório de Referência em Esquistossomose do Instituto Aggeu Magalhães. A cepa utilizada foi a LE (Leandro Evangelista).

2.3 Grupos e protocolos de tratamento

Os grupos experimentais totalizaram 90 camundongos, estes foram divididos de acordo com a(s) droga(s) e o tipo de tratamento utilizado (tabela 01).

Os protocolos de tratamentos adotados foram: a) Praziquantel (VETRANAL lote BCCD7939, Sigma-Aldrich, USA) a 50 mg/kg/5 dias consecutivos por gavagem; b) P-MAPA (lote S20, Farmabrasilis, BRA) 100mg/kg/dose única via intraperitoneal; c) e Silimarina (S0292 lote BCBT9170 contendo silibina em ≥30%, Sigma-Aldrich, USA) a 10 mg/kg a cada 48h por 10 doses, via gavagem. Somado a esses protocolos, também foram utilizadas combinações dessas drogas para o tratamento, como mostrado na tabela 01.

Tabela 01 – Grupos experimentais infectados e doses terapêuticas

GRUPOS	Nº de animais
G1 – Controle: sem tratamento	06
G2 – 50mg/kg de Praziquantel	12
G3 – 100mg/kg de P-MAPA	12
G4 – 50mg/kg + 100mg/kg Praziquantel + P-MAPA	12
G5 – 10mg/kg de Silimarina	12
G6 – 10 mg/kg + 50mg/kg Silimarina + Praziquantel	12
G7 – 10 mg/kg + 100mg/kg Silimarina + P-MAPA	12
G8 – 50mg/kg + 100mg/kg + 10mg/kg Praziquantel + P-MAPA + Silimarina	12

Fonte: Elaborado pela autora (2021).

As drogas foram diluídas em Dimetilsulfóxido (DMSO) (Lote SHBK0789 Sigma-Aldrich, USA) com tampão fosfato-salino (PBS) 1x a 3% para o Praziquantel, 4,5% para o P-MAPA e 1% para a Silimarina, respectivamente.
Os tratamentos começaram 60 dias após a infecção, com as drogas administradas em diferentes momentos quando combinadas (Quadro 01).

Quadro 01 – Cronograma de aplicação das drogas

GRUPOS	DROGAS	INICIO APLICAÇÃO	EUTANÁSIA
G2	PZQ	61dpi	68dpi
G3	MAPA	61dpi	68dpi
G4	PZQ MAPA	61dpi 61dpi	68dpi
G5	SILI	61dpi	83dpi
G6	SILI PZQ	61dpi 61dpi	83dpi
G7	SILI MAPA	61dpi 76dpi	83dpi
G8	PZQ MAPA SILI	61dpi 76dpi 61dpi	83dpi

Legenda: dpi = dias pós infecção; PZQ = Praziquantel; MAPA = P-MAPA; SILI = Silimarina.
Fonte: Elaborado pela autora (2021).

2.4 Eutanásia

As eutanásias ocorreram três dias após o fim dos tratamentos por aprofundamento anestésico utilizando xilazina 10mg/kg e cloridrato de cetamina 150mg/kg. O procedimento foi realizado por um veterinário.

2.5 Contagem e classificação de ovos

Para vias de contagem total de ovos, intestino e fígado foram digeridos em hidróxido de potássio (KOH) a 4% à temperatura ambiente de acordo com o método descrito por Cheever e Anderson (Cheever e Anderson, 1971).

Quanto à classificação, o oograma avaliou três fragmentos da porção média do intestino delgado para o estudo da viabilidade dos ovos (maduros, imaturos e mortos) (Pellegrino e Faria, 1965).

2.6 Recuperação de vermes

Os vermes foram recuperados através de perfusão sanguínea com solução de perfusão (citrato de sódio (Na3C6H5O7) a 7,5% + cloreto de sódio (NaCl) a 15,0%) e filtrados em papel filtro. Além da contagem através do papel filtro, os vermes obtidos nos órgãos também foram contabilizados.

2.7 Análise Estatística

Os dados foram analisados utilizando o software GraphPad Prism 5, (versão 5.02) e foram expressos como média e desvio padrão da média. As análises de tamanho de granuloma foram feitos utilizando o software Image J (versão 1.53n).

3 RESULTADOS

Na tabela 02, todos os grupos com silimarina apresentaram perdas expressivas. Os grupos G3 e G4 não apresentaram nenhum óbito durante o tratamento.

Tabela 02 – Grupos experimentais e números de animais antes e após o tratamento

GRUPOS	Nº de animais iniciais	Nº de animais finais
G1 – Controle doente infectado	06	06
G2 – Praziquantel	12	10
G3 – P-MAPA	12	12
G4 – Praziquantel + P-MAPA	12	12
G5 – Silimarina	12	06
G6 – Silimarina + Praziquantel	12	06
G7 – Silimarina + P-MAPA	12	08
G8 – Praziquantel + P-MAPA + Silimarina	12	06

Fonte: Elaborado pela autora (2021).

Na Tabela 03, observa-se o quantitativo total de ovos recuperados pós-digestão em KOH a 4% dos tecidos hepático e intestinal, após a porção para histologia ser separada. O G6 e o G8 apresentaram os menores quantitativos de ovos recuperados. O G3 e G4 obtiveram quantitativos maiores que o Controle infectado. Já no intestino, o G3, G5 e G7 foram maiores que o controle infectado.

Tabela 03 – Quantitativo total de ovos no fígado e intestino

GRUPO	TOTAL DE OVOS	
	FÍGADO	INTESTINO
G1 – Controle Infectado	240.905	19.110
G2 – Praziquantel	184.100	12.985
G3 – P-MAPA	300.790	32.515
G4 – Praziquantel + P-MAPA	266.945	17.780
G5 – Silimarina	123.510	35.820
G6 – Silimarina + Praziquantel	39.030	7.560
G7 – Silimarina + P-MAPA	169.320	51.930
G8 – Praziquantel + P-MAPA +Silimarina	73.830	13.680

Fonte: Elaborado pela autora (2021).

3.1 Quantitativo de vermes recuperados por grupo

Como mostrado na Tabela 04, os grupos G3, G5 e G7 se destacaram com os maiores quantitativos de vermes recuperados. O G6 e o G8 apresentaram os menores quantitativos de vermes recuperados.

Tabela 04 – Quantitativo de vermes recuperados

GRUPOS	VERMES		
	MACHO	FÊMEA	CASAL
G1	05	04	10
G2	02	01	06
G3	04	00	48
G4	01	00	02
G5	03	01	16
G6	00	00	01

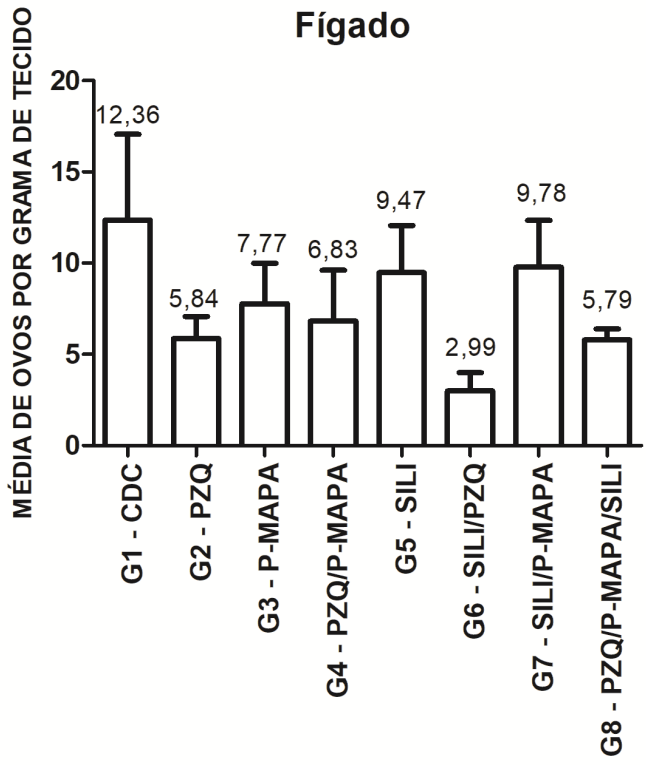
G7	01	02	30
G8	01	01	04

Fonte: Elaborado pela autora (2021).

3.2 Efeitos in vivo do Praziquantel, P-MAPA e Silimarina, isolados e combinados, no número de ovos/g de tecido de fígado.

O G6, tratado com praziquantel e silimarina, apresentou a maior redução de ovos totais (Figura 03) e da média de ovos por grama de tecido (2,99) no fígado quando comparado aos outros grupos. O G5 e o G7 apresentaram as maiores médias de ovos por grama de tecido hepático, além do grupo controle (Figura 03).

Figura 03 – Média de ovos/g de fígado sob ação dos diferentes tratamentos



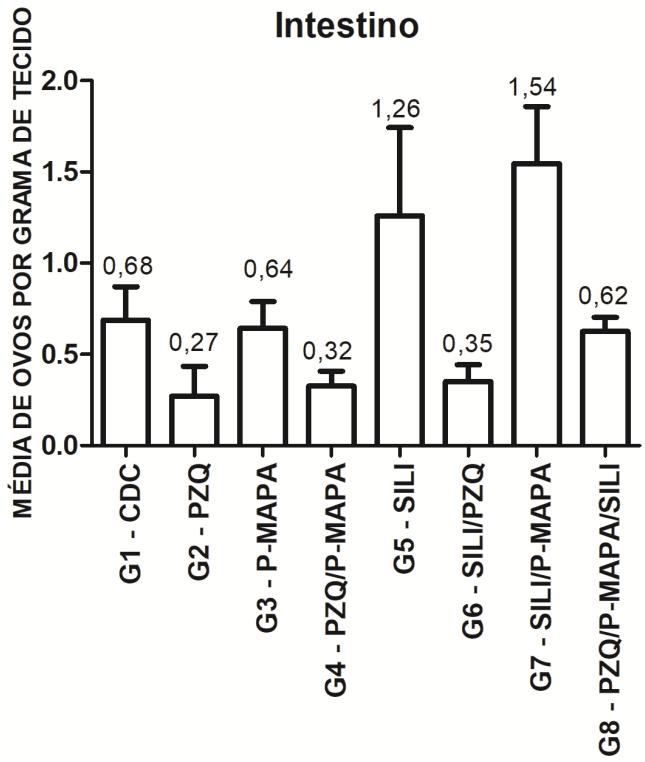
Legenda: CDC= Controle doente crônico; PZQ = Praziquantel; PZQ/P-MAPA= Praziquantel + P-MAPA; SILI = Silimarina; SILI/PZQ= Silimarina + praziquantel; SILI/P-MAPA= silimarina + P-MAPA; PZQ/P-MAPA/SILI= praziquantel + P-MAPA + silimarina

Fonte: Elaborado pela autora (2021)

3.3 Efeitos in vivo do Praziquantel, P-MAPA e Silimarina, isolados ou combinados, no número de ovos/g de tecido de intestino.

O G2, G4 e G6 apresentaram os menores valores de média de ovos por grama de tecido no intestino. Os grupos G5 e G7 apresentaram as maiores médias de ovos/g de tecido intestinal e os maiores valores na contagem total de ovos (Figura 04) e o G6 apresentou a menor média de ovos no intestino.

Figura 04 – Cheever de Intestino



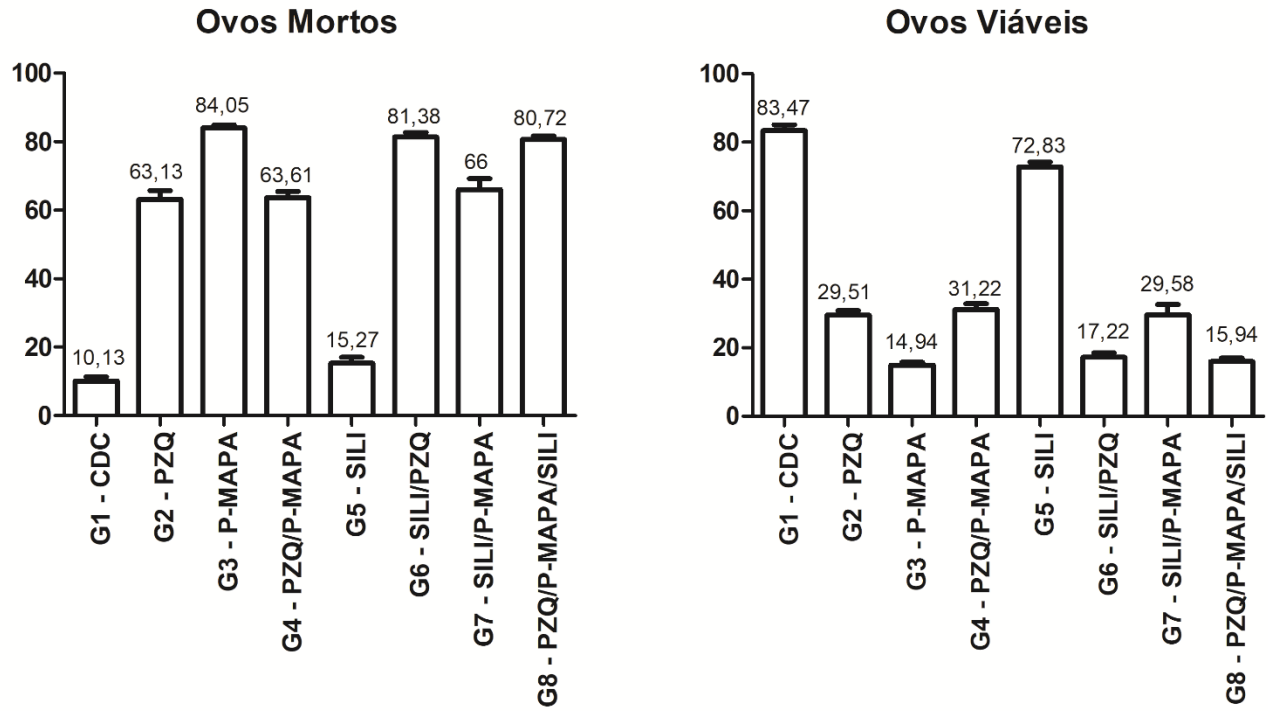
Legenda: CDC= Controle doente crônico; PZQ = Praziquantel; PZQ/P-MAPA= Praziquantel + P-MAPA; SILI = Silimarina; SILI/PZQ= Silimarina + praziquantel; SILI/P-MAPA= silimarina + P-MAPA; PZQ/P-MAPA/SILI= praziquantel + P-MAPA + silimarina

Fonte: Elaborado pela autora (2021)

3.4 Efeitos in vivo do Praziquantel, P-MAPA e Silimarina, isolados ou combinados, na viabilidade de ovos de S.mansoni (Oograma)

Os padrões de oviposição foram mais altos nos grupos G3, G4, G7, G8, porém, apesar da alta quantidade de ovos, mais de 80% dos ovos estavam inviáveis, ou seja, mortos. O G5 foi o único grupo com um maior quantitativo de ovos viáveis, além do controle (Figura 05).

Figura 05 – Oograma de ovos viáveis e mortos



Legenda: CDC= Controle doente crônico; PZQ = Praziquantel; PZQ/P-MAPA= Praziquantel + P-MAPA; SILI = Silimarina; SILI/PZQ= Silimarina + praziquantel; SILI/P-MAPA= silimarina + P-MAPA; PZQ/P-MAPA/SILI= praziquantel + P-MAPA + silimarina

Fonte: Elaborado pela autora (2021)

3.5 Quantitativo e viabilidade de ovos no intestino após o tratamento com Praziquantel, P-MAPA e Silimarina, isolados ou combinados.

A tabela 05 mostra que o G4, G6 e G8 apresentaram as maiores porcentagens de ovos mortos no intestino, chegando a 83,91%.

Tabela 05 – Comparativo de média de ovos

GRUPOS	MÉDIA DE OVOS/ GRAMA DE TECIDO	MORTOS (%)
G1	0,68	10,29
G2	0,27	62,31
G3	0,32	63,61
G4	0,64	83,91
G5	1,26	15,28
G6	0,35	81,39
G7	1,54	66,95
G8	0,62	80,73

Fonte: Elaborado pela autora (2021)

4 DISCUSSÃO

Desde a descoberta do praziquantel na década de 1970, este tem sido o fármaco de escolha no tratamento contra a esquistossomose, juntamente com oxamniquine, droga que entrou em desuso devido ao seu alto custo, além do surgimento de resistência (CHEVALIER et al., 2016). A falta de mais opções terapêuticas contra a esquistossomose é preocupante devido a ameaça de surgimento de casos de resistência ao Praziquantel (STELMA et al., 1995).

Os ovos de Schistosoma mansoni desempenham um importante papel na patogênese da esquistossomose. Após a oviposição, aproximadamente metade dos ovos são eliminados pelo hospedeiro através das fezes para, então, dar continuidade ao ciclo (SCHWARTZ; FALLON, 2018). A outra metade dos ovos se espalha pelos tecidos, mais comumente no fígado e intestino, desencadeando uma resposta imunológica que é mediada pelas células Th2, formando granulomas ao redor dos ovos nos tecidos (CHIARAMONTE et al., 1999).

Estudos sugerem a associação de medicamentos para o tratamento contra a esquistossomose a fim de potencializar a atividade anti-helmíntica e ovicida do praziquantel, reduzir às ameaças de resistência à droga (KATZ, COELHO, 2008; MELMAN et al., 2009; SILVA et al., 2020), além de tratar as consequências provocadas pela doença e diminuir os danos causados nos tecidos (EL-HAWARY et al., 2018). Nossos achados mostram que os grupos de animais tratados com praziquantel tiveram os menores números de casais de vermes recuperados e, quando associado a silimarina, o número foi ainda menor, com apenas um casal de verme recuperado.

Durante o nosso estudo, os grupos tratados com silimarina, isolada ou combinada, sofreram os maiores quantitativos de óbitos. Este resultado pode ter relação com o longo período de duração do protocolo de tratamento adotado para a silimarina, além do intervalo esperado para que os camundongos atingissem a fase crônica da doença, que totalizaram 83 dias até a realização da eutanásia, enquanto o protocolo de eutanásia adotado para os demais grupos de animais foi de 68 dias após a infecção.

O grupo de animais tratado com P-MAPA isolado apresentou um aumento superior a 300% no quantitativo de casais de verme recuperados quando comparado ao grupo controle doente. Contudo, contrariando nossos achados, um estudo realizado por Silva et al. (2021), cujo objetivo foi avaliar a ação do P-MAPA na esquistossomose, mostra que o P-MAPA foi capaz de reduzir o quantitativo de vermes recuperados em 50% em relação ao grupo controle doente.

Em relação à contagem total de ovos, o grupo tratado com silimarina + praziquantel apresentou uma redução de mais de 83% no quantitativo de ovos no tecido do fígado quando comparado ao grupo controle infectado, o que pode ser justificado pela quantidade baixa de vermes recuperados nesse grupo, que foi apenas um casal.

Em um estudo realizado por El-Lakkany et al. (2012), que investigou os efeitos anti-inflamatórios e antifibróticos da silimarina e do praziquantel na fibrose hepática esquistossomótica, o grupo tratado com praziquantel + silimarina obteve um resultado similar e sofreu redução de aproximadamente 70% dos ovos no tecido hepático. Apesar de El-Lakkany et al. (2012) terem utilizado a silimarina em uma dosagem 75x maior do que a do nosso protocolo de tratamento, alcançando uma redução de aproximadamente 40% dos ovos no fígado no grupo tratado com silimarina isolada, nossos resultados foram similares e a silimarina foi capaz de reduzir mais de 50% dos ovos no tecido hepático.

Já no tecido intestinal, o grupo tratado com praziquantel + silimarina reduziu cerca de 60% dos ovos em relação ao grupo controle doente. Em um estudo feito por El-Hawary et al. (2018), utilizando Capparis spinosa L., praziquantel e silimarina, o grupo tratado com praziquantel + silimarina foi capaz de reduzir em 100% a média de ovos no tecido intestinal. Neste mesmo estudo, o grupo tratado com praziquantel isolado obteve uma redução de 97% dos ovos no intestino, o que difere dos nossos resultados, no qual o grupo tratado com praziquantel isolado reduziu apenas 32% dos ovos no tecido intestinal.

Estes resultados sugerem que a Silimarina pode potencializar a atividade ovicida e anti-helmíntica do Praziquantel quando associados, assim como no estudo realizado por El-lakkany et al., (2012), no qual o efeito potencializador foi atribuído às propriedades i) antioxidantes da silimarina, eliminando os produtos das reações oxidativas, e ii) imunomoduladoras, auxiliando na destruição imunomediada de vermes e ovos. Somado a isso, os autores afirmam que a silimarina demonstrou ser uma promissora ferramenta terapêutica no tratamento da fibrose hepática desencadeada pela esquistossomose, já que possui atividade regeneradora, hepatoprotetora e anti-inflamatória.

Em nosso estudo, o grupo tratado com P-MAPA isolado sofreu um aumento de mais de 25% no quantitativo total de ovos no fígado quando comparado ao grupo controle. Este resultado difere dos achados de Silva et al. (2021), que obtiveram uma redução de quase 70% dos ovos no tecido hepático no grupo tratado com P-MAPA quando comparado ao grupo controle doente. Apesar dos resultados de ambos estudos terem sido com a dosagem de 100mg/kg do P-MAPA, Silva et al. (2021) optaram pelo tratamento de 3 dias de administração da droga, enquanto o

nosso estudo utilizou dose única. Esta diferença no tratamento pode ter sido responsável pela desigualdade no resultado de cada estudo.

O grupo tratado com P-MAPA isolado obteve um aumento de 70% no número de ovos no intestino, ao contrário da pesquisa realizada por Silva et al. (2021), onde houve redução de quase 90% dos ovos no intestino dos camundongos tratados com P-MAPA. Esta diferença pode ser explicada pela alta quantidade de casais de verme recuperados em nosso estudo (48 casais). O número de casais de verme existentes no hospedeiro interfere diretamente no quantitativo de ovos depositados no organismo e, consequentemente, a serem alojados nos tecidos e, por conseguinte, no resultado do tratamento.

No oograma, o grupo tratado com praziquantel + P-MAPA apresentou 83,91% dos ovos mortos, diferente dos achados de Silva et al (2021), que apresentaram apenas 40% dos ovos mortos no grupo tratado com P-MAPA + praziquantel. Essa diferença nos resultados pode ter relação com a dosagem de P-MAPA utilizada pelos autores neste grupo, que foi 5mg/kg. Em relação ao grupo silimarina + praziquantel, nosso estudo obteve 81,93% dos ovos mortos, já El-Hawary et al. (2018), com achados semelhantes aos nossos, obtiveram 98,33% dos ovos de Schistosoma mansoni mortos. Além disso, em nossa pesquisa, o grupo tratado com silimarina + praziquantel + P-MAPA também atingiu resultados positivos, neste obteve-se 80,73% dos ovos mortos, indicando uma possível sinergia entre essas drogas.

Por último, é importante salientar que a principal limitação em nossa discussão se baseia no fato que Silva et al. (2021) publicaram o primeiro e único estudo que avalia os efeitos terapêuticos do P-MAPA isolado e combinado ao praziquantel na infecção causada por Schistosoma mansoni. Além do mais, não há estudos publicados que avaliem a interação do P-MAPA, praziquantel e silimarina combinados, o que dificulta a discussão desses dados.

4 CONCLUSÃO

A silimarina isolada, apesar de não conseguir alterar de forma significativa a oviposição, demonstrou bons resultados quando associada ao praziquantel, potencializando seu efeito anti-helmíntico e ovicida. O praziquantel e o P-MAPA, isolados ou combinados, foram capazes de interferir na oviposição, levando a um aumento do quantitativo de ovos inviáveis. A combinação das três drogas também demonstrou resultados positivos, o que pode indicar uma promissora ferramenta terapêutica contra a esquistossomose e os danos causados pela doença. Novos testes com dosagens e durações diferentes se fazem necessários para que o mecanismo de ação e sinergia entre as drogas sejam compreendidos de forma precisa.

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ANEXO A – NORMAS DE SUBMÍSSÃO DA REVISTA CIENTÍFICA

TABLE OF CONTENTS

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DESCRIPTION

Experimental Parasitology emphasizes modern approaches to **parasitology**, including **molecular biology** and **immunology**. The journal features original research papers on the physiological, metabolic, immunologic, biochemical, nutritional, and chemotherapeutic aspects of **parasites** and **host-parasite relationships**.

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Cancer Research UK, 1975. Cancer statistics reports for the UK. http://www.cancerresearchuk.org/ aboutcancer/statistics/cancerstatsreport/ (accessed 13 March 2003). Reference to a dataset:

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